Editorial

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Deciphering the highway code for lymphocyte traffic along the gut – liver axis

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The mucosal immune compartment of the gut functions as a barrier for microbes and other extrinsic agents that might potentially harm the host [1]. Due to its anatomical position at the confluence of portal and arterial blood, the liver functions as a second barrier for gut-derived pathogens and microbial products [2]. It is believed that this close functional link between gut and liver could explain associations between inflammatory diseases of gut and liver [1].

The most striking example is primary sclerosing cholangitis (PSC) that is marked by portal liver inflammation and bile duct obstruction; more than 80% of these patients suffer from ulcerative colitis as a comorbidity [1]. One explanation for this pathogenic link could be aberrant homing of mucosal lymphocytes to the liver [1]. Indeed, gut-primed T cells were detected in livers of PSC patients [3] and, in a recent murine study, gut-activated CD8 T cells were found to induce antigen-specific cholangitis [4]. Conversely, there is evidence that T cells primed in the liver might influence mucosal immune responses in the gut, as CD4 T cells primed by liver sinusoidal endothelial cells (LSECs) have been shown to acquire a gut-homing phenotype [5]. The pathogenic significance and the rules of the proposed mutual T cell traffic along the gut-liver axis are thus far not clear.

The present study by Eickmeier *et al.* in this issue of the *Journal of Hepatology* provides a systematic analysis of the distinct migration and activation patterns of CD8 T cells that were either primed in the gut or in the liver. By adoptive transfer of naïve ovalbumin (OVA)-specific OT-I cells to transgenic mice displaying gut- or liver-restricted expression of OVA, they generated gut- or liver-primed CD8 T cells *in vivo*. The differential migration behaviour of gut- or liver-activated OT-I T cells was then investigated in secondary recipients. Confirming previous reports that priming in the gut-associated lymphoid tissue (GALT) confers gut tropism to lymphocytes [6], the authors found that gut-activated CD8 T cells preferentially accumulated in the small intestine, rather than in lymphoid tissues. Moreover, gut-activated OT-I T cells were also found to migrate to the livers of recipient mice, as has been already recently reported by the same group [4]. Of note, although cognate antigen-expression in the liver of recipient mice further increased hepatic accumulation of gut-activated CD8 T cells, homing to the liver was observed irrespective of hepatic antigen-presentation. These findings indicate that liver infiltration by gut-primed CD8 T cells seems to occur by way of antigen-independent bystander hepatitis. However, the induction of liver pathology was only observed in mice featuring hepatic OVA-expression [4]. Apart from the observed liver tropism of gut-activated CD8 T cells, the present study showed that liver-primed CD8 T cells did not accumulate in the gut, suggesting that liver-activated CD8 T cells are not involved in intestinal immune responses.

To further explore the different imprints of gut and liver on activation and migration of CD8 T cells, Eickmeier *et al.* performed transcriptome analysis of gut- or liver-activated CD8 T cells that were isolated from mesenteric lymph nodes or from the liver after *in vivo* priming. In line with previous reports that intestinal activation by CD103+ dendritic cells (DCs) confers gut tropism to T cells [6], gut-activated, but not liver-activated or naïve OT-I T cells displayed significant co-expression of the gut-homing markers CCR9 and $\alpha4\beta7$, explaining the observed exclusion of liver-primed CD8 T cells from the gut. However, in the present study, hepatic antigen expression was mainly confined to hepatocytes and it remains to be seen whether priming by other liver antigen presenting cell types might result in diverse migratory behaviors. Although stellate cells and liver dendritic cells were not able to induce CCR9 and $\alpha4\beta7$ expression in CD8 T cells [3], it is conceivable that LSECs might be able to induce such phenotype in CD8 T cells, as this was recently shown for LSEC-primed CD4 T cells [5].

In the present study, gut- or liver-specific priming of CD8 T cells occurred under non-inflammatory conditions, however, it is most likely that an inflammatory context during priming greatly influences the activation status and migration behavior of CD8 T cells. Indeed, proinflammatory cytokines or microbial products greatly increase production of T cell-attracting chemokines and adhesion molecules [1], and promote maturation of antigen-presenting cells [7]. In fact, hepatic upregulation of CCL25 and MAdCAM, the respective binding partners for CCR9 and $\alpha4\beta7$, has been observed in inflamed livers of PSC patients [8]. In contrast, Eickmeier *et al.* did not observe hepatic expression of CCL25 and MAdCAM here, presumably due to the absence

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of hepatic inflammation in their model. The question then is how hepatic accumulation of gut-derived CD8 T cells occurred, notwithstanding the absence of CCL25 and MAdCAM? One might speculate that additional chemokine ligands such as CXCR3 ligands or integrins that allow binding to ICAM-1 and VCAM-1 [9] could be responsible for hepatic transendothelial migration of gut-primed CD8 T cells under non-inflammatory conditions. Nonetheless, further studies are needed to investigate how inflammation at the priming site or in the target organ can influence enterohepatic T cell activation and migration.

Confirming their previous findings, Eickmeier *et al.* found that gut-primed CD8 T cells display an activated phenotype. Liver-primed CD8 T cells, however, showed a distinct phenotype with markers of activated (CD44), naïve (CD62L), and central memory cells (Ly6C). Interestingly, liver-activated T cells also expressed Neuropilin-1 (Nrp-1). Note that a similar Nrp-1+ central-memory like phenotype was recently found to be induced in naïve CD8 T cells by liver sinusoidal endothelial cells (LSECs) [10]. These LSEC-primed memory CD8 T cells can acquire effector function upon antigen-specific re-stimulation in an inflammatory environment [10]. Thus, although intrahepatic priming of CD8 T cells usually results in tolerance [11] or an apoptosis-prone phenotype [12], it is conceivable that LSEC-primed memory CD8 T cells might re-encounter their cognate antigen in the gut and might thereby contribute to mucosal immunity. The recent finding that LSECs can confer a gut-homing phenotype to CD4 T cells [4] seems to support this notion. It remains to be seen, whether autoimmune liver diseases might result from inflammatory reactivation in the gut of long-lived memory cells recognizing antigens present in the liver. Indeed, a study by the Adams group, showing a memory-phenotype of CCR9+ T cells in PSC livers, seems to point in this direction [13].

In addition to the analysis of enterohepatic CD8 T cell migration, future studies should also address the migration patterns of CD4 T cells. Gut-primed CD4 T cells can induce hepatitis in IL10-deficient mice after *Trichinella spiralis* infection [14]. Moreover, liver infiltration with Th17 cells has been found in patients suffering from diverse autoimmune liver diseases [15]. Since the gut is a prime site of Th17 induction in response to bacterial stimulation [16], it seems likely that gut-derived Th17 cells might contribute to hepatic inflammation.

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Conflict of interest

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